FEMS Microbiology Letters 250 (2005) 97–104

FEMS MICROBIOLOGY Letters

www.fems-microbiology.org

Diverse pathogenicity of *Burkholderia cepacia* complex strains in the *Caenorhabditis elegans* host model

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Received 5 May 2005; received in revised form 23 June 2005; accepted 24 June 2005

First published online 13 July 2005

Edited by C. Winstanley

Abstract

A fast screening method was developed to assess the pathogenicity of a diverse collection of environmental and clinical *Burkholderia cepacia* complex isolates in the nematode *Caenorhabditis elegans*. The method was validated by comparison with the standard slow-killing assay. We observed that the pathogenicity of *B. cepacia* complex isolates in *C. elegans* was strain-dependent but species-independent. The wide range of observed pathogenic phenotypes agrees with the high degree of phenotypic variation among species of the *B. cepacia* complex and suggests that the taxonomic classification of a given strain within the complex cannot predict pathogenicity.

Keywords: *Burkholderia cepacia* complex; *Caenorhabditis elegans*; Host model; Pathogenicity screening

1. Introduction

The genus *Burkholderia* comprises a diverse group of Gram negative microorganisms that thrive in different ecological niches including soil, water, the rhizosphere, and humans. In particular, the *Burkholderia cepacia* complex consists of ten closely related species or genomovars that can be isolated from both environmental and clinical sources [1]. Usually non-pathogenic for healthy individuals, *B. cepacia* complex isolates cause a variety of infections in immunocompromised patients, and in patients with chronic granulomatous disease (CGD) and cystic fibrosis (CF) [1]. Two species of the *B. cepacia* complex, *B. multivorans* (formerly genomovar II) and *B. cenocepacia* (formerly genomovar III), account for the majority of isolates from CF patients in North America [2,3] and Europe [4]. *B. cepacia* complex isolates differ in utilization of carbon sources, susceptibility to antibiotics [5–7], and the presence of a pathogenicity island [8]. Also, individual differences among strains from the same species have been detected in amoeba [9], murine [10] and plant infection models [11].

The nematode *Caenorhabditis elegans*, lacking adaptive immunity, is a useful host model for studying innate immune responses to bacterial pathogens [12,13]. This model is genetically tractable from the perspectives of

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0378-1097/$22.00
doi:10.1016/j.femsle.2005.06.050

both host and pathogen, and thus, serves to investigate evolutionary conserved mechanisms of microbial pathogenesis and innate immunity [14–17]. A previous study employed a small number of the *B. cepacia* complex strains and demonstrated that they can cause infection in *C. elegans* [18], and the infection-like process was characterized using the *B. cenocepacia* strain H111 [19]. However, a systematic analysis of the pathogenic diversity within *B. cepacia* complex species has not been performed in this model. In this study, we have developed a rapid screening method to characterize in *C. elegans* the pathogenic phenotypes of a representative number of environmental and clinical *B. cepacia* complex isolates. We observed a wide range of pathogenic phenotypes and demonstrate that the pathogenicity of *B. cepacia* complex isolates in *C. elegans* is strain-dependent but species-independent.

2. Materials and methods

2.1. Bacterial and nematode strains

Caenorhabditis elegans Bristol N2 and DH26 strains were obtained from the *Caenorhabditis* Genetics Center, University of Minnesota, Minneapolis. A collection of *B. cenocepacia* K56-2 transposon mutants with survival defects in the rat lung model of infection [20] was used to develop the 48-well plate mortality assay. A subset of these mutants is listed in Table 1. Strains belonging to the *B. cepacia* complex are listed in Table 2. All species were represented by at least three isolates with the exception of one single strain of *B. ubonensis* (genomovar X). Most of the strains from the *B. cepacia* complex experimental strain panel [21,22] were also included in the study. When available, information on the geographic and biological sources of the isolates was included in Table 2. Most isolates were obtained from North America and Europe. A few strains were obtained from Argentina, Australia, Senegal and Vietnam.

2.2. Forty-eight-well plate mortality assay

Forty-eight-well plates containing 600 μl of NG agar [19] were inoculated with 20 μl of overnight bacterial cultures, incubated at 37 °C for 3 h, and then again overnight at room temperature to allow the formation of a bacterial lawn. Five to ten hypochlorite-synchronized L4 larvae of *C. elegans* strain Bristol N2 were deposited onto each well and incubated at 20 °C. The percentage of live worms and their morphological appearance was registered after two days. The total number of nematodes including the parental worms (if still alive) and the progeny nematodes (if any) was scored after five days. The non-pathogenic *Escherichia coli* OP50 strain was used as a negative control. From preliminary experiments, comparing infections with *E. coli* OP50 and *B. cenocepacia* K56-2, we established that a given strain of the *B. cepacia* complex was pathogenic for *C. elegans* if one of the following criteria was met: (i) a sick appearance at day 2, which included reduced locomotive capacity and the presence of distended intestine; (ii) percentage of live worms at day $2 \leqslant 50\%$; and (iii) total number of worms at day $5 \leqslant 50$. The presence of any one, two or three of these criteria was scored as 1, 2, and 3, respectively, differentiating mild from severe infections (Tables 1 and 2). Any given strain was considered pathogenic when at least one criterion was observed (pathogenicity score 1, 2 or 3). Conversely, a strain was considered non-pathogenic when no symptoms of disease were observed during the course of the infection experiment (pathogenicity score 0).

2.3. Slow-killing assay

Slow-killing assays were performed using the *C. elegans* strain DH26 [23]. Six-well plates containing 5 ml of NG agar [19] were inoculated with 50 μl of the overnight cultures adjusted to an OD_{600} of 1.7 and incubated 24 h at 37 °C to allow the formation of a bacterial lawn. Twenty to forty hypochlorite-synchronized L4 larvae of *C. elegans* strain DH26 were inoculated to each plate and incubated at 25 °C. Each assay was performed in triplicate. Plates were scored for live and dead worms every 24 h. For each killing assay nematode survival was calculated by the Kaplan–Maier method, and survival differences were tested for significance by the use of the log rank test (GraphPad Prism, version 4.0).

3. Results and discussion

3.1. Validation of the 48-well plate mortality assay

To validate the 48-well plate mortality assay in the *C. elegans* model as a rapid method for screening large numbers of strains, we exploited our collection of transposon mutants in *B. cenocepacia* strain K56-2, which display reduced survival rates in the rat agar bead model of lung infection [20]. We reasoned that these mutants would elicit a wide range of pathogenic phenotypes in the *C. elegans* model, thus, facilitating the assessment of the pathogenicity criteria. These parameters were based on the percent survival and appearance of worms at two days post-infection, and the total number of parental and progeny nematodes after five days, as described in Section 2. As controls, we performed infections with the non-pathogenic *E. coli* strain OP50 (data not shown) and the parental *B. cenocepacia* K56-2 whose pathogenic phenotypes were assigned scores of 0 and 3, respectively. From the 75 mutants screened,

Table 1
Comparison between the 48-well plate mortality assay and the slow-killing assay using selected *B. cenocepacia* K56-2 transposon mutants

Strain	Known or putative function of the mutated gene[a]	48-Well plate mortality assay of *C. elegans* Bristol N2 strain				Slow-killing assay of *C. elegans* DH26		
		Appearance at day 2[b]	% of live worms at day 2	Total number of worms at day 5	PS[c]	% of live worms at day 2	Median survival (days)[d]	*P*-value[e]
4A7	*paaE*, ferredoxin reductase	Normal	100	100–500	0	97	Undefined	<0.0001
3A3	Cation efflux pump	Normal	100	50–100	0	71	3	<0.0001
10F1	Hypothetical protein	Normal	90	50–100	0	73	3	<0.0001
16H8	*ugpB*, glycerol 3-P binding periplasmic protein	Normal	72	50–100	0	71	3	<0.0001
28D9	Translation initiator inhibitor *tdcF* and *yjgF*	Sick	66	50–100	1	96	3	<0.0001
36B4	*cpxA*, capsular polysaccharide export ATP-binding protein	Sick	100	50–100	1	69	3	<0.0001
38E2	*hemK*, methyltransferase	Sick	100	50–100	1	83	3	<0.0001
33H3	*wbiI*, epimerase/dehydratase	Sick	50	20–50	2	60	3	<0.0001
34A1	Transcriptional regulator	Sick	50	10–20	2	27	2	0.5059 NS
28D8	UTP-glucose-1-phosphate	Sick	40	0	3	20	2	0.2162 NS
20D2	D-lactate-dehydrogenase/oxidoreductase	Sick	47	0	3	34	2	0.3695 NS
K56-2	Parental strain	Sick	22	0	3	24	2	–
OP50	*E. coli* feeding strain	Normal	100	50–100	0	100	Undefined	<0.0001

NS, non-significant.

[a] As described by Hunt et al. [20].

[b] The appearance of worms was scored as sick when impaired locomotion or a distended intestine was found.

[c] Pathogenicity score 0, no signs of disease; 1, 2 and 3, one two or three symptoms of disease, respectively (see Section 2).

[d] Obtained from Kaplan–Meier survival plots (see Fig. 1).

[e] *P* values calculated from pair wise comparisons (log rank test) by each transposon mutant strain versus K56-2 wild-type strain.

Table 2
Pathogenicity of *B. cepacia* complex strains in *C. elegans* Bristol N2

Species	GV[a]	Strain	Comments	Appearance at day 2	% of live worms at day 2	Number of worms at day 5	PS[b]
B. cepacia	I	ATCC 25416	Onion, U.S.A., Bccesp[c]	Sick	67	50–100	1
		CEP0509	CF, Australia, Bccesp	Normal	95	>500	0
		FC124	CGD, Canada	Sick	94	50–100	1
		MC353	Environmental (onion)	Sick	8	0	3
		MC76	Environmental (onion)	Sick	13	0	3
		MC81	Environmental (onion)	Sick	17	20–50	3
B. multivorans	II	249-2	Laboratory, U.S.A., Bccesp	Normal	100	50–100	0
		ATCC 17616	Soil, U.S.A., Bccesp	Normal	100	100–500	0
		C0514	CF, sputum, Canada	Normal	100	100–500	0
		C3430	CF, sputum, Canada	Normal	93	100–500	0
		C4297	CF, sputum, Canada	Sick	88	50–100	1
		C5274	CF, sputum, Canada	Normal	100	50–100	0
		C5393	CF, Canada, Bccesp	Normal	73	100–500	0
		C5568	CF, sputum, Canada	Sick	66	50–100	1
		CEP0108	CGD, Lung, U.S.A.	Normal	100	100–500	0
		CEP484	CF, U.S.A.	Normal	100	100–500	0
		FC0147	CGD, Canada	Normal	100	100–500	0
		FC0442	CGD, blood, U.S.A.	Normal	100	100–500	0
		LMG 16660	CF, U.K., Bccesp, Glasgow epidemic strain	Normal	100	100–500	0
		LMG 16665	brain abscess, U.K.	Normal	100	100–500	0
		CEP1016	CF, U.K.	Sick	ND	0	2
		CEP1017	CF, U.K.	Normal	80	100–500	0
		CEP1018	U.K.	Normal	100	100–500	0
		CEP1019	CF, Ireland	Sick	88	20–50	2
B. cenocepacia	III	BC7	CF, Canada, Bccesp	Normal	85	50–100	0
		C1484	CF, Canada	Sick	29	0	3
		C3865	CF, Canada	Sick	92	0	2
		C4455	CF, Canada	Sick	100	>500	1
		C5424	CF, Canada, Bccesp	Normal	98	100–500	0
		CEP024	CF, U.S.A., Bccesp	Normal	84	50–100	0
		CEP0931	CGD, endotracheal tube, U.S.A.	Sick	55	0	2
		CEP1067	CGD, blood, Canada	Sick	28	0	3
		CEP511	CF, Australia, Bccesp	Normal	90	100–500	0
		CEP054	CF, U.S.A.	Normal	100	50–100	0
		CP 706-J	CF, U.S.A.	Sick	60	50–100	1
		F28368-82	CF, Canada	Sick	31	20–50	3
		F38192-89	CF, Canada	Sick	44	0	3
		FC0127	CGD	Sick	92	20–50	2
		H111	CF	Sick	33	0	3
		J2315	CF, U.K., Bccesp	Normal	97	20–50	1
		K56-2	CF, Canada, Bccesp	Sick	22	0	3
		L10	CF, Canada	Sick	57	0–10	2
		PC 527-I	CF, U.S.A.	Sick	30	0	3
		PC 701-J	CF, U.S.A.	Sick	78	0–10	2
B. stabilis	IV	C6061	CF, sputum, Canada	Normal	57	50–100	0
		CEP0559	CF, Canada	Normal	78	50–100	0
		FC0473	CF, Belgium	Normal	100	50–100	0
		LMG 14086	respirator, U.K., Bccesp	Sick	45	10–20	3
		LMG 14294	CF, Belgium, Bccesp	Normal	80	100–500	0
		LMG 18870	CF, Canada, Bccesp	Normal	74	50–100	0
		LMG 18888	Human blood, Belgium, Bccesp	Sick	46	0–10	3
B. vietnamiensis	V	C2822	CF, sputum, Canada	Normal	81	100–500	0
		FC0369	Rhizosphere, Vietnam	Normal	100	50–100	0
		FC0441	CGD, Canada	Sick	46	>500	2
		CCUG 31370	CF, Sweden. Bccesp	Normal	100	100–500	0
		LMG18835	CF, U.S.A., Bccesp	Sick	100	100–500	1
B. dolosa	VI	CEP021	CF, U.S.A.	Normal	96	100–500	0
		CFLG	CF, Argentina	Normal	92	50–100	0
		L6	CF, Canada	Sick	81	10–20	2

Table 2 (*continued*)

Species	GV[a]	Strain	Comments	Appearance at day 2	% of live worms at day 2	Number of worms at day 5	PS[b]
		LMG 18943	CF, U.S.A.	Sick	64	50–100	1
		LMG 21443	*Alysicarpus glumaceus*, root nodule, Senegal, Bccesp	Sick	100	0–10	2
		LMG 21820	CF, U.K., Bccesp	Normal	87	20–50	1
B. ambifaria	VII	LMG 17828	Corn roots, U.S.A., Bccesp	Sick	57	0	2
		LMG 19182	pea rhizosphere, U.S.A., Bccesp	Sick	83	0–20	2
		LMG 19467	CF, Australia, Bccesp	Sick	4	0	3
B. anthina	VIII	LMG 16670	*Carludovica palmata*, rhizosphere. U.K., Bccesp	Sick	36	0	3
		LMG 20980	Soil rhizosphere, U.S.A., Bccesp	Sick	100	10–20	2
		LMG 20983	CF, sputum, U.K., Bccesp	Sick	75	0	2
		LMG 21821	CF, U.S.A., Bccesp	Sick	91	0–10	2
B. pyrrocinia	IX	LMG 14191	Soil, Fujisawa Pharm. Co. Bccesp, Patent strain	Sick	49	0–10	3
		LMG 21822	Cornfield soil, U.S.A., Bccesp	Sick	28	0	3
		LMG 21823	Water, U.S.A., Bccesp	Sick	17	0	3
		LMG 21824	CF, U.K., Bccesp	Sick	40	0	3
B. ubonensis	X	LMG 20358	Surface soil, Thailand. Bccesp	Sick	10	0	3

Comparable results were obtained in at least two independent experiments.

[a] Genomovar.

[b] Pathogenicity score as described in Section 2.

[c] *B. cepacia* complex experimental strain panel.

we found 31 (41%) with a pathogenicity score of 3, comparable to that of the parental K56-2 strain. This suggested that the mutated genes in these strains had no effect in attenuating the infection in *C. elegans*. In contrast, 44 mutants displayed various levels of attenuation, including 33 mutants with pathogenicity score 2, five mutants with pathogenicity score 1, and 6 mutants with pathogenicity score 0.

To compare the 48-well plate mortality assay with the more established slow-killing assay, we selected a subset of the transposon mutants with different pathogenicity scores (Table 1), which were employed in killing assays using the *C. elegans* DH26 strain [23]. This nematode strain has a temperature sensitive mutation in the spermatogenesis *fer-15* gene. As worms are sterile at 25 °C, it is possible to count the original worms for longer periods of time after infection without the interference of progeny worms. The rate of killing by *B. cenocepacia* K56-2 on *C. elegans* DH26 at 25 °C and on *C. elegans* Bristol N2 at 20 °C was indistinguishable (data not shown). The *B. cenocepacia* K56-2 mutants examined in *C. elegans* DH26 also exhibited different degrees of pathogenicity (Fig. 1) that generally correlated well with the results of the 48-well plate mortality assay (Table 1). The transposon mutants with pathogenicity scores 0 and 1 in the 48-well killing assay were highly attenuated with respect to the K56-2 wild-type strain when tested in the slow-killing assay (P-value < 0.0001). While the mutant 4A7 did not show any significant nematode killing activity over a 5-day period, *C. elegans* infected with mutants 3A3, 10F1, 16H8, 28D9, 36B4, and 38E2 displayed a median survival of three days (Table 1). In contrast, mutants 28D8 and 20D2 (pathogenicity score 3) were as pathogenic as K56-2 (Table 1). The mutant 33H3 (pathogenicity score 2) showed an attenuated phenotype (P-value < 0.0001 and median survival of three days) in the slow-killing assay. However, the percent of live worms at day 2 was lower than the rest of the transposon mutants that also showed an attenuated phenotype and had pathogenicity scores 0 and 1. The only exception to this correlation was the mutant 34A1, which showed a pathogenicity score 2 in the 48-well mortality assay but was not significantly different from K56-2 in the slow-killing assay. This disparity could be due to an overestimation in the number of counted worms at day 2 in the 48-well plate mortality assay. Therefore, we conclude from these observations that the 48-well plate mortality assay correlates with the slow-killing assay. Further studies are underway in our laboratory to characterize in detail the function of the mutated genes in relation to infection in *C. elegans*.

3.2. Pathogenicity of clinical and environmental B. cepacia complex strains in the C. elegans model

The pathogenicity of representative strains from the *B. cepacia* complex was screened with the 48-well plate mortality assay. *B. cepacia* (genomovar I) was represented by six strains, four environmental isolates and two clinical strains isolated from CF and CGD patients. The

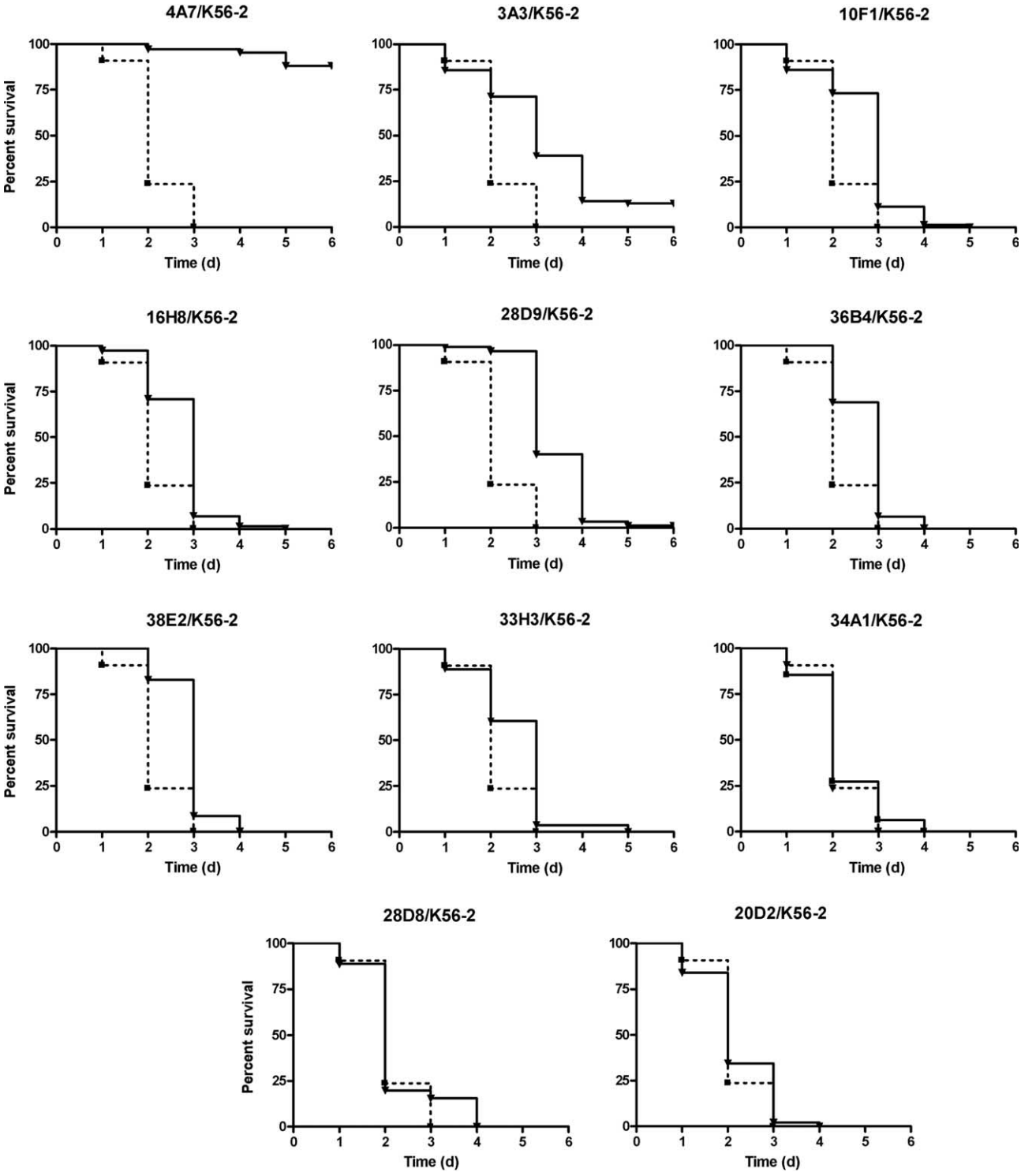

Fig. 1. Kaplan–Meier survival plots of selected transposon mutants. The killing ability of wild-type *B. cenocepacia* K56-2 strain ($n = 76$) was compared with that of the STM-mutants 4A7 ($n = 109$), 3A3 ($n = 77$), 10F1 ($n = 71$), 16H8 ($n = 72$), 28D9 ($n = 87$), 36B4 ($n = 45$), 38E2 ($n = 35$), 33H3 ($n = 109$), 34A1 ($n = 110$), 28D8 ($n = 71$) and 20D2 ($n = 93$) in slow-killing assay experiments using *C. elegans* DH26 strain. *n*, Number of worms at day 0. Dashed lines with squares, *B. cenocepacia* K56-2 strain; solid lines with triangles, any given transposon mutant of K56-2 strain (indicated in the title of each graphic).

pathogenic phenotypes for these isolates were not homogenous, ranging from the non-pathogenic CEP0509 to the highly pathogenic strains MC353, MC76 and MC81. Fourteen of the 18 *B. multivorans* (genomovar II) strains were non-pathogenic. Two isolates, C4297 and C5568 caused a mild infection in *C. elegans* while strains

CEP1016 and CEP1019 were the most pathogenic. *B. cenocepacia* (genomovar III) strains were highly diverse with respect to their pathogenic phenotypes. Even the strains K56-2, J2315 and BC7, which are considered to be clonal [21], exhibited different pathogenic phenotypes. Strain K56-2 had the highest degree of pathogenicity while J2315 only caused a slight decrease in the number of progeny worms. This could be attributed to the differences in O-antigen expression between strains K56-2 and J2315 [24]. However, reconstitution of O-antigen production in J2315 by complementation did not render the strain more pathogenic in *C. elegans* (data not shown). Hence, the differences between these two clonal strains in the pathogenicity for *C. elegans* might be explained by differences other than O-antigen production. Five of the seven *B. stabilis* (genomovar IV) isolates were highly attenuated, while the degree of pathogenicity of isolates from *B. vietnamiensis* (genomovar V) and *B. dolosa* (genomovar VI) was diverse. All isolates from *B. ambifaria* (genomovar VII) *B. anthina* (genomovar VIII), *B. pyrrocinia* (genomovar IX), and *B. ubonensis* strain LMG20358 were pathogenic for *C. elegans*. But we cannot conclude that all the strains from these species are pathogenic given that we only had a limited number of strains available for this study.

The distribution of pathogenic and non-pathogenic strains according to their genomovar classification is summarized in Fig. 2. On average, the proportion of strains having a pathogenic phenotype was highest in *B. cepacia* and *B. cenocepacia*, while *B. multivorans* and *B. stabilis* strains were the least pathogenic (Fig. 2). Environmental isolates appeared to be more pathogenic for *C. elegans* than the clinical isolates (78% of the environmental isolates and 52% of the human disease isolates). However, it cannot be concluded that the clinical and environmental isolates necessarily differ in their capacity to cause disease in humans. More likely, the observed differences may reflect the adaptation of clinical strains to the lung environment with the concomitant loss of other characteristics required for colonization and infection of *C. elegans*.

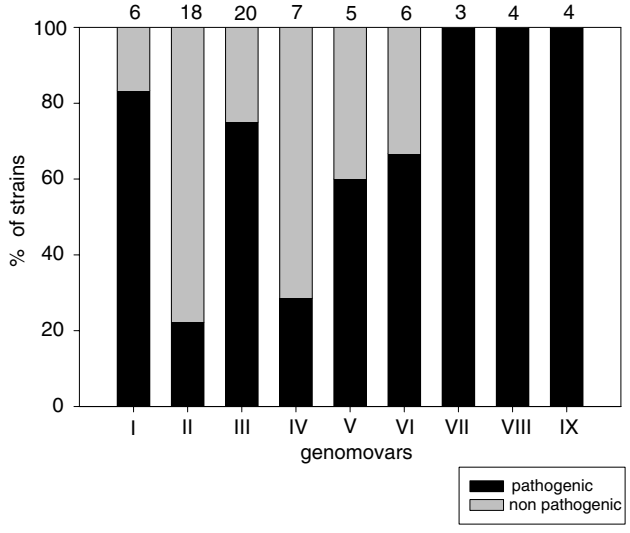

Fig. 2. Distribution of pathogenicity of *B. cepacia* complex isolates in the *C. elegans* Bristol N2 model. The strains shown in Table 2 were grown in NG agar plates at 37 °C and the 48-well mortality assay was performed. Percent of pathogenic and non-pathogenic strains was calculated for each genomovar (see Section 2). Numbers over the bars represent the total number of isolates in each genomovar.

4. Conclusions

In this study, we demonstrate that *B. cepacia* complex strains show a great diversity of pathogenic phenotypes for *C. elegans*. This variability, which also applies to strains within the same species, could reflect either loss or acquisition of accessory genetic material, which may provide functional diversity among individual strains. Therefore, the assignment of an environmental or clinical strain as a given species or genomovar does not predict the potential risk for infection. We also identified *B. cenocepacia* K56-2 transposon mutants that were non-pathogenic in both nematodes and rats. Thus, at least some of the survival-associated properties of *B. cenocepacia* are common to both nematodes and mammalian hosts, as it has been demonstrated for *Pseudomonas aeruginosa* [14,25]. A detailed analysis at the molecular level of the pathogenicity of *B. cepacia* complex strains in *C. elegans* will provide additional clues to better understand the adaptation of these microbes to multiple environments.

Acknowledgments

We thank M. Köthe for technical help with the *C. elegans* model, D. Henry and P. Vandamme for assisting with the classification of genomovar isolates, S. Thyssen for assisting with the statistical analysis, and Karen Keith for critical reading of the manuscript. S.T.C. was supported by a Postdoctoral Fellowship Award from the Canadian Cystic Fibrosis Foundation. This study was supported by operating grants from the Canadian Institutes of Health Research and the Canadian Cystic Fibrosis Foundation. M.A.V. holds a Canada Research Chair in Infectious Diseases and Microbial Pathogenesis.

References

[1] Coenye, T. and Vandamme, P. (2003) Diversity and significance of *Burkholderia species* occupying diverse ecological niches. Environ. Microbiol. 5, 719–729.

[2] Speert, D.P., Henry, D., Vandamme, P., Corey, M. and Mahenthiralingam, E. (2002) Epidemiology of *Burkholderia cepacia*

complex in patients with cystic fibrosis, Canada. Emerg. Infect. Dis. 8, 181–187.
[3] LiPuma, J.J., Spilker, T., Gill, L.H., Campbell, P.W., Liu, L. and Mahenthiralingam, E. (2001) Disproportionate distribution of *Burkholderia cepacia* complex species and transmissibility markers in cystic fibrosis. Am. J. Respir. Crit. Care Med. 164, 92–96.
[4] Mahenthiralingam, E., Baldwin, A. and Vandamme, P. (2002) *Burkholderia cepacia* complex infection in patients with cystic fibrosis. J. Med. Microbiol. 51, 533–538.
[5] Vermis, K., Vandamme, P.A. and Nelis, H.J. (2003) *Burkholderia cepacia* complex genomovars: utilization of carbon sources, susceptibility to antimicrobial agents and growth on selective media. J. Appl. Microbiol. 95, 1191–1199.
[6] Henry, D.A., Mahenthiralingam, E., Vandamme, P., Coenye, T. and Speert, D.P. (2001) Phenotypic methods for determining genomovar status of the *Burkholderia cepacia* complex. J. Clin. Microbiol. 39, 1073–1078.
[7] Nzula, S., Vandamme, P. and Govan, J.R.W. (2002) Influence of taxonomic status on the in vitro antimicrobial susceptibility of the *Burkholderia cepacia* complex. J. Antimicrob. Chemother. 50, 265–269.
[8] Baldwin, A., Sokol, P.A., Parkhill, J. and Mahenthiralingam, E. (2004) The *Burkholderia cepacia* epidemic strain marker is part of a novel genomic island encoding both virulence and metabolism-associated genes in *Burkholderia cenocepacia*. Infect. Immun. 72, 1537–1547.
[9] Marolda, C.L., Hauröder, B., John, M.A., Michel, R. and Valvano, M.A. (1999) Intracellular survival and saprophytic growth of isolates from the *Burkholderia cepacia* complex in free-living amoebae. Microbiology 145, 1509–1517.
[10] Chu, K.K., Davidson, D.J., Halsey, T.K., Chung, J.W. and Speert, D.P. (2002) Differential persistence among genomovars of the *Burkholderia cepacia* complex in a murine model of pulmonary infection. Infect. Immun. 70, 2715–2720.
[11] Bernier, S.P., Silo-Suh, L., Woods, D.E., Ohman, D.E. and Sokol, P.A. (2003) Comparative analysis of plant and animal models for characterization of *Burkholderia cepacia* virulence. Infect. Immun. 71, 5306–5313.
[12] Ewbank, J.J. (2002) Tackling both sides of the host-pathogen equation with *Caenorhabditis elegans*. Microb. Infect. 4, 247–256.
[13] Aballay, A. and Ausubel, F.M. (2002) *Caenorhabditis elegans* as a host for the study of host-pathogen interactions. Curr. Opin. Microbiol. 5, 97–101.
[14] Tan, M.W., Mahajan-Miklos, S. and Ausubel, F.M. (1999) Killing of *Caenorhabditis elegans* by *Pseudomonas aeruginosa* used to model mammalian bacterial pathogenesis. Proc. Natl. Acad. Sci. USA 96, 715–720.
[15] Labrousse, A., Chauvet, S., Couillault, C., Kurz, C.L. and Ewbank, J.J. (2000) *Caenorhabditis elegans* is a model host for *Salmonella typhimurium*. Curr. Biol. 10, 1543–1545.
[16] Sifri, C.D., Begun, J., Ausubel, F.M. and Calderwood, S.B. (2003) *Caenorhabditis elegans* as a model host for *Staphylococcus aureus* pathogenesis. Infect. Immun. 71, 2208–2217.
[17] Kurz, C.L., Chauvet, S., Andres, E., Aurouze, M., Vallet, I., Michel, G.P., Uh, M., Celli, J., Filloux, A., De Bentzmann, S., Steinmetz, I., Hoffmann, J.A., Finlay, B.B., Gorvel, J.P., Ferrandon, D. and Ewbank, J.J. (2003) Virulence factors of the human opportunistic pathogen *Serratia marcescens* identified by in vivo screening. Embo J. 22, 1451–1460.
[18] O'Quinn, A.L., Wiegand, E.M. and Jeddeloh, J.A. (2001) *Burkholderia pseudomallei* kills the nematode *Caenorhabditis elegans* using an endotoxin-mediated paralysis. Cell. Microbiol. 3, 381–393.
[19] Kothe, M., Antl, M., Huber, B., Stoecker, K., Ebrecht, D., Steinmetz, I. and Eberl, L. (2003) Killing of *Caenorhabditis elegans* by *Burkholderia cepacia* is controlled by the cep quorum-sensing system. Cell. Microbiol. 5, 343–351.
[20] Hunt, T.A., Kooi, C., Sokol, P.A. and Valvano, M.A. (2004) Identification of *Burkholderia cenocepacia* genes required for bacterial survival in vivo. Infect. Immun. 72, 4010–4022.
[21] Mahenthiralingam, E., Coenye, T., Chung, J.W., Speert, D.P., Govan, J.R., Taylor, P. and Vandamme, P. (2000) Diagnostically and experimentally useful panel of strains from the *Burkholderia cepacia* complex. J. Clin. Microbiol. 38, 910–913.
[22] Coenye, T., Vandamme, P., LiPuma, J.J., Govan, J.R. and Mahenthiralingam, E. (2003) Updated version of the *Burkholderia cepacia* complex experimental strain panel. J. Clin. Microbiol. 41, 2797–2798.
[23] Roberts, T.M. and Ward, S. (1982) Membrane flow during nematode spermiogenesis. J. Cell Biol. 92, 113–120.
[24] Ortega, X., Hunt, T.A., Loutet, S., Vinion-Dubiel, A.D., Datta, A., Choudhury, B., Goldberg, J.B., Carlson, R. and Valvano, M.A. (2005) Reconstitution of O-specific lipopolysaccharide expression in *Burkholderia cenocepacia* strain J2315, which is associated with transmissible infections in patients with cystic fibrosis. J. Bacteriol. 187, 1324–1333.
[25] Tan, M.W., Rahme, L.G., Sternberg, J.A., Tompkins, R.G. and Ausubel, F.M. (1999) *Pseudomonas aeruginosa* killing of *Caenorhabditis elegans* used to identify *Pseudomonas aeruginosa* virulence factors. Proc. Natl. Acad. Sci. USA 96, 2408–2413.